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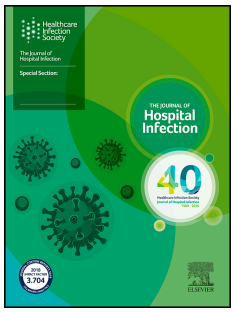


Gargling with povidone iodine has a short-term inhibitory effect on SARS-Cov-2 in COVID-19 patients

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# Gargling with povidone iodine has a short-term inhibitory effect on SARS-Cov-2 in COVID-19 patients

**Running title: Gargling effect with PVP-I on SARS-Cov-2**

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It is known that povidone iodine (PVP-I) solutions have virucidal action against severe acute respiratory syndrome coronavirus (SARS-CoV) *in vitro* [1,2,3]. In this study, the saliva of coronavirus disease 2019 (COVID-19) patients was collected up to 2 hours after PVP-I gargling and the dynamics of SARS-CoV-2 infectivity in saliva were assessed by a real-time reverse transcription–polymerase chain reaction (rRT–PCR) and determination of the infectious viral load.

Patients (aged ≥ 20 years) who had symptoms indicative of SARS-CoV-2 infection within the last 7 days or asymptomatic patients with a cycle threshold < 40 for SARS-CoV-2 ribonucleic acid (RNA), as determined by rRT–PCR of saliva, were included in this study (n=35). Patients who had an iodine allergy or thyroid disease were excluded. This study was approved by the Institutional Review Board (Hokkaido University Hospital Division of Clinical Research Administration Number: 020-0111), and written informed consent was obtained from all participants.

Baseline saliva samples were collected prior to intervention with iodine. Then, patients rinsed their mouths for 20 seconds with 20 mL of PVP-I gargle solution (Meiji Co., Ltd, Tokyo, Japan), which was diluted 15 times with water. Patients repeated gargling with PVP-I three times, then rinsed their mouths with water. After gargling, saliva was collected at four time points: immediately after gargling, and 30, 60, and 120 minutes (min) later. Patients collected saliva samples themselves by spitting into a sterile cup (PP Screw cup 50; ASIAKIZAI Co., Tokyo, Japan). Viral RNA was quantified in the samples by RT–PCR and the virus was titrated in cultured cells.

For RT–PCR, 200 μL of saliva was added to 600 μL of PBS, mixed vigorously, then centrifuged at 20,000 × *g* for 5 min at 4°C, and 140 μL of the supernatant was used as the sample. rRT-PCR was conducted in accordance with the manual for the Detection of

Pathogen 2019-nCoV Ver. 2.9.1. (https://www.niid.go.jp/niid/images/lab-manual/2019-nCoV20200319.pdf). Total RNA was extracted using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) and rRT-PCR was performed using the QuantiTect Probe RT–PCR Kit (QIAGEN) in the QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA). The sequences of the primers and TaqMan probe used for detection of the SARS-CoV-2 genome were as follows: forward primer (NIID_2019 -nCOV_N_F2, 5′ AAATTTTGGGGACCAGGAAC 3′), reverse primer (NIID_2019-nCOV_N_R2, 5′ TGGCAGCTGTGTAGGTCAAC 3′), TaqMan probe (NIID_2019-nCOV_N_P2, 5′ FAM-ATGTCGCGCATTGGCATGGA-BHQ 3′).

Viral titers were determined as the 50% tissue culture infective dose ($TCID_{50}$) of the virus. Vero E6 cells expressing the type II transmembrane serine protease (Vero-TMPRSS2) [4] were seeded into 96-well plates and incubated with a serial dilution of patient saliva. Three days later, cytopathic effects were examined. The samples in which infectious SARS-CoV-2 was detected before PVP-I gargling (i.e., $> 10 \times TCID_{50}$ of the virus) were targeted in this study.

Of a total of 35 COVID-19-positive patients, 24 were excluded from the study because they had undetectable SARS-CoV-2 RNA or a viral titer of $< 10 \times TCID_{50}$ in their baseline saliva sample. Thus, 11 patients were analyzed in this study. The average viral RNA copies and viral titers were compared at each time point using the Wilcoxon rank sum test. The p-value threshold for significance was set at $< 0.05$.

Figure 1A shows the change in viral RNA copies ($log_{10}$ copies/mL) after PVP-I gargling. A statistically significant decrease in viral RNA was observed in the samples taken immediately after gargling and at 30 and 60 min, compared with before gargling.

Figure 1B shows the change in viral titer ($log_{10}$ $TCID_{50}$/mL) after PVP-I gargling. A statistically significant decrease was observed in viral titer immediately after gargling and at 60 min, compared with before gargling. The viral titers in the samples at 30 min showed no significant difference ($p = 0.055$), but the median value was lower compared with the samples taken before gargling.

In conclusion, viral copies and titers were significantly decreased 60 min after gargling. The reason for the temporary increase in viral titer at 30 min may become clear as the number of examined cases increases. However, importantly, our data indicated that PVP-I gargling effectively suppressed SARS-CoV-2 infectivity in saliva for 60 min. The application of PVP-I may be an effective measure to reduce the infection risk in situations such as during dental treatment and oral examination by physicians.

We recognize a limitation of our study is that this study was performed under simple conditions to minimize the risk of infection, and was carried out without a control group for gargling just with water. Despite the limitation, our findings support the use of PVP-I gargling in preventing infections via saliva over a short period.

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**Conflicts of Interest**

None declared.

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1 None.

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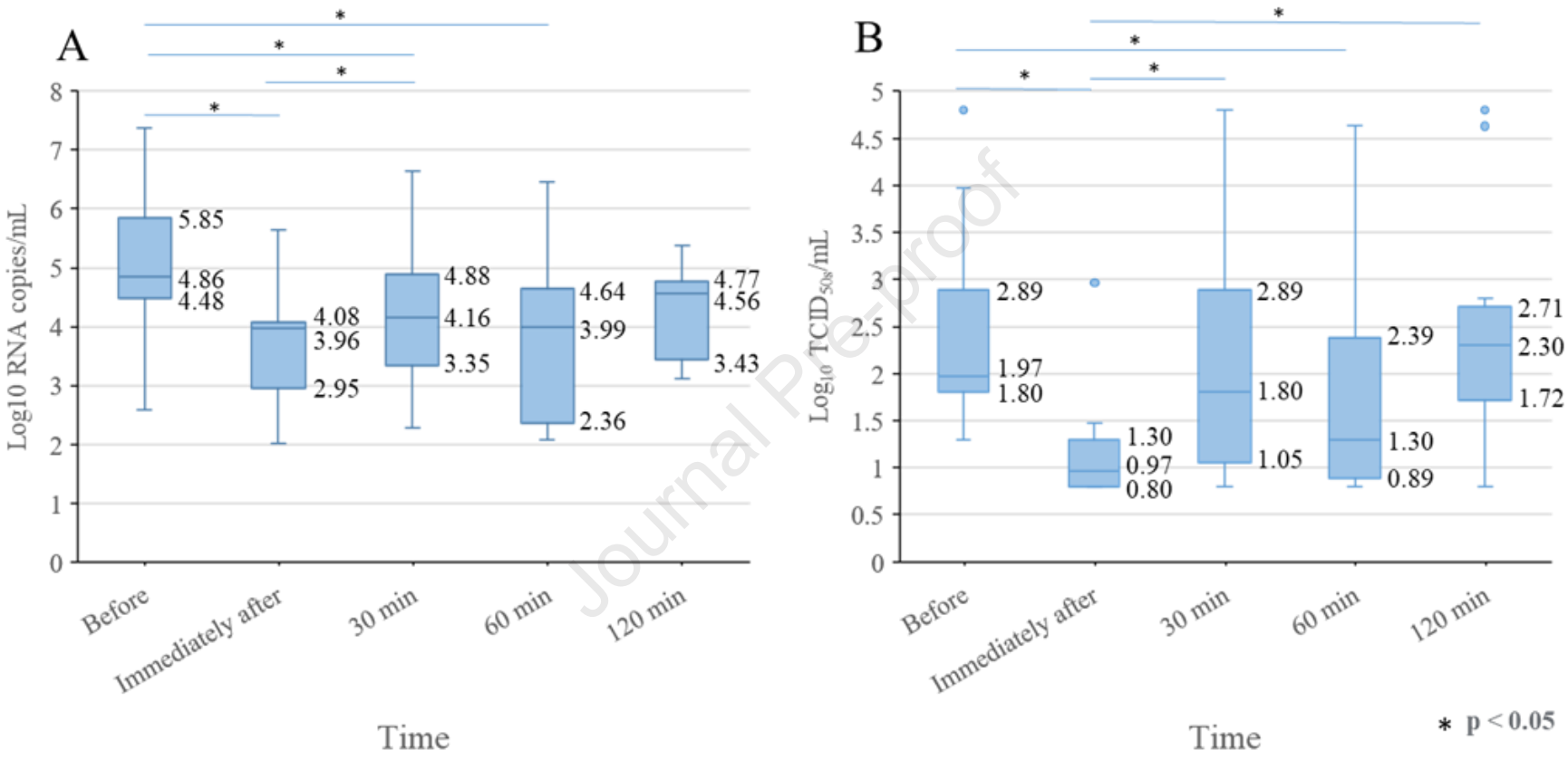


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**Figure Legend**

Figure 1. Changes in the SARS-COV-2 RNA level (A) and viral titer (B) in saliva samples before and after povidone iodine gargling

Box plots (median, interquartile range, 5th and 95th percentile). SARS-CoV-2: severe acute respiratory syndrome coronavirus 2. TCID50: tissue culture infectious dose. RNA: ribonucleic acid. Before: before gargling, Immediately after, immediately after gargling, min: minutes after gargling.